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Research Strategies for Behavioral Teratology Studies

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Several compelling aruguments have been advanced in support of expanding the use of "behavioral teratology" evaluations as routine components of toxicologic screening procedures. As a basis for development of effective behavioral teratology screening approaches, a conceptual framework is presented which interrelates: (1) changes in relative functional brain capacity with age, (2) possible times and durations of exposures to environmental insults, and (3) various types of toxicity testing procedures carried out at appropriate time points in relation to different exposure periods. Within that context, several research strategies for behavioral teratology studies are concisely posed and evaluated. These include: (1) clinical hypothesis testing, where particular effect(s) of a given agent are evaluated based on hypotheses derived from clinical or epidemiological observations; (2) comprehensive screening approaches, where multifaceted, long-term longitudinal neurobehavioral evaluations are employed to assess whether any of a large number of possible deletarious effects are exerted by an agent and at what threshold exposure levels; (3) alternative screening heuristics, by which adequate assessments of neurobehavioral toxicity of various agents may be accomplished without completion of more exhaustive, but also more expensive and time-consuming comprehensive screening protocols.

Introduction

Considerable interest has developed in recent years among toxicologists and other biomedical specialists in regard to the evaluation of neurobehavioral effects of known or suspected toxic agents. The growth of interest in "behavioral toxicology" research has been stimulated in part by extensive documentation, accumulated over the past 10-20 years, of numerous examples of deleterious neurological and behavioral effects associated with exposure of humans and animals to various environmental agents and drugs, as reviewed in several relevant symposium volumes (*1-3*). Research described in the above volumes and other published reports have also contributed to calls in Congress (*4*) for the enactment of federal statutes requiring behavioral assessments as components of future toxicity screening procedures. Importantaly for the present discussion, among the recommendations under consideration are ones concerning the postnatal evaluation of toxic effects on behavior following environmental insults early in development, i.e., during prenatal or early postnatal periods.

Several arguments have been advanced as justification for expanding neurobehavioral toxicity screening efforts in general and for behavioral teratology screening in particular as an area of special importance. It has been noted, for example, that "Since behavior represents an integrated response of the organism, an impairment in the functioning of nearly any system may be reflected as indicators that some, as yet covert, toxic action has occurred" (*5*). That significant behavioral changes may occur at exposure levels for some agents insufficient to produce histologically detectable damage to the central nervous system or other organs is suggested by studies such as those on hyper-

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vitaminosis A reviewed by Butcher as part of the present session (*6*). To the extent that behavioral changes do occur sooner on a dose-response curve for toxic effects than other signs of toxicity, e.g., anatomical abnormalities, then deviations from normal behavior patterns may comprise an "early warning system" (*7*) presaging more severe and overtly obvious damage induced by an agent at higher doses or after longer exposure periods. Aside from serving as early warning signals, undesirable behavioral effects in and of themselves may constitute a substantial cost associated with exposure to a particular agent, particularly if irreversible severe behavioral deficits are induced by the agent. Both of these arguments are compelling points supporting the need for expanded behaviroal toxicology screening efforts. As for more specifically including behavioral teratology—studies as a regular component of such screening procedures, it has been argued (*5,8,9*), on the basis of strong evidence, that the developing nervous system may be particularly vulnerable to the damaging effects of many agents. This is perhaps most dramatically epitomized to date by the unfortunate consequences of exposures to low levels of methylmercury during pregnancy, as outlined in descriptions of the insiduous nature of Minamata Disease and its counterpart induced in experimental animal models (*9*).

Given that convincing arguments can be made for expanding behavioral toxicology research in general and behavioral teratology efforts in particular, many problems remain concerning how to accomplish this goal most effectively, especially if such evaluations are to be routinely conducted as part of standardized toxicity screening procedures. As of yet, no single clear-cut answer or definitive approach has yet emerged that can be recommended without reservations. Most alternatives are beset by their own special difficulties or disadvantage, as well as by frequent common problems centering around immense monetary, space, and personnel commitments, plus lengthy time spans necessary to carry them through to decisive outcomes. Nevertheless, much pertinent information does exist in the neurobiology, behavioral toxicology, psychopharmacology, and experimental/developmental psychology literature that can be called upon in an effort here to pose briefly and evaluate several possible alternative research strategies for conducting behavioral teratology assessments.

Defining the Scope of the Problem

How one conceptualizes the overall dimensions of a particular problem obviously often determines much about the alternatives that might be contemplated for dealing with it. In this case, constructing even a very general conceptual framework concerning the likely "biology" of potential exposure problems and expected time courses of associated pathological processes aids greatly in defining the full scope of the problem at hand and provides hints as to possible approaches needed to deal with it.

The key concept upon which the present theoretical framework is based is what will be termed here "functional brain capacity." It is by no means a completely new concept, nor is the present use of the concept in relation to behavioral toxicology problems novel. Kety (*10*), in 1956, as described by Weiss and Simon (*11*), estimated the rate of normal decline with age of the functional capacity of the brain based on changes in various indices of brain function. These included, for example, decreases in cerebral blood flow, cerebral oxygen consumption, and cortical neuron density as a function of age. Weiss and Simon (*11*) plotted the normal expected decline in functional capacity, as defined by neuronal cell density and predicted by Kety's calculations, in relation to chronological age, using 25 years as a base of 100% capacity. They then went on to extrapolate from Kety's figures and to plot graphically estimated accelerated rates of decline in functional capacity if a hypothetical environmental insult at age 25 induced even very small excess rates (of 0.1% to 1.0% excess/year) of cell loss. As the Weiss and Simon (*11*) plots well illustrate, the anticipated cummulative effect on functional capacity over many years of even such small excess rates of cell loss can be expected to be quite substantial. For example, with just 0.5% excess cell loss per year from age 25, the expected functional capacity at chronological age 55 can be projected to be reduced to a level roughly equivalent to that which would have been seen at 70 years if a normal rate of decline in neuronal density had occurred.

As indicated by Weiss and Simon (*11*), their calculations were not meant to represent a precise model of any specific known environmental exposure situation or its consequences. Nevertheless, assessment of changes

in the functional capacity of the brain or subsystems of it would appear to be the key theoretical issue implicitly addressed by most studies of neuro-behavioral toxicity. In view of this, consideration here of possible effects on functional brain capacity of hypothetical environmental insults of varying durations and occurring at different stages of life will be used as a basis for subsequent discussion of different behavioral teratology research approaches.

For present purposes, the concept of functional brain capacity should be explicitly broadened to reflect not just such indices as cerebral blood flow and neuronal cell density in the brain. Rather, it is defined here as also reflecting the number or density of functionally intact neurons in the entire central nervous system (CNS) and its peripheral extensions, the number or density of functionally intact, nonaberrant synaptic connections therein, and the intactness of various associated neurochemical processes, e.g., neurotransmitter synthesis, release, and degradation. All of these are factors determining the maximum complexity of neurobehavioral processes capable of being mediated by the nervous system of an organism and the reserve capacity available to it to sustain such processes under challenging circumstances. A hypothetical curve representing net relative levels of functional brain capacity, determined by balancing off the above factors at different periods of life for most mammalian species, is incorporated in the overall conceptual scheme illustrated in Figure 1.

In regard to the normal course of changes in functional capacity thought to occur over time after conception, as represented by the curve at the top of Figure 1, functional capacity is seen here as steadily rising prenatally and neonatally. This reflects the rapid proliferation of neural tissue during early development and the establishment of functional synaptic contacts as the brain matures. In regard to the former process, i.e., cell proliferation, data on actual periods of maximum proliferation of neurons comprising different regions in the brain of the rat and mouse are summarized in this session's paper by Rodier (*12*) and indicate that neural proliferation in some brain areas continues for some time after birth. As the proliferation process ends, however, functional brain capacity probably begins to reach as asymptote, likely sometime not long after puberty, and is seen in the present scheme as leveling off for a prolonged period during adulthood. This is based on the likely offsetting of the effects of continuous normal cell deaths by gains in processing efficiency, e.g., as would be expected to occur as new synaptic connections are laid down and other changes result from "learning" or experience. Later in life, functional brain capacity is shown to decline, starting at some point in old age or "geriatric" life period. This decline in functional capacity occurs when the accumulated effects of cell losses and other harmful shifts in neural function begin to predominate and lead to the manifestation of neurobehavioral deficits defined as "senility" or "senescence."

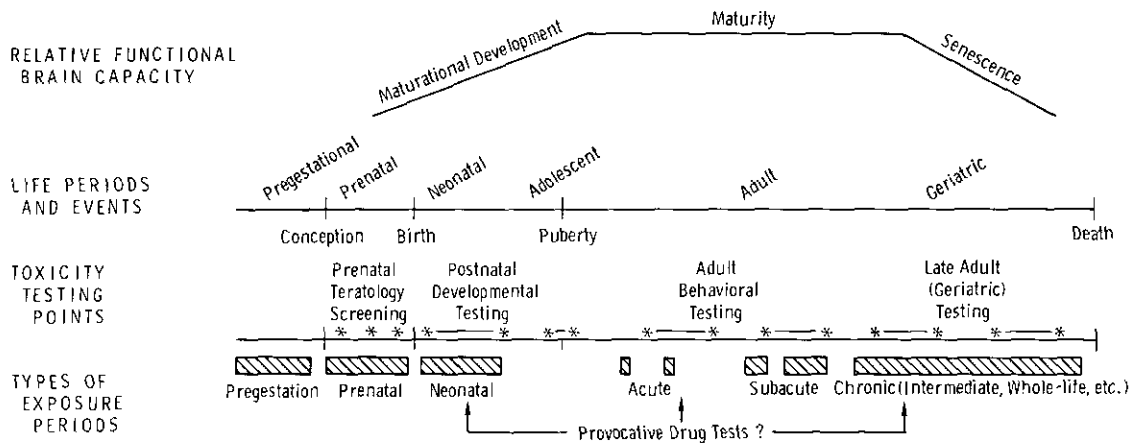

FIGURE 1. Schematic representation of conceptual framework upon which are based suggestions for longitudinal designs for behavioral teratology studies. Normal changes in relative levels of functional brain capacity, determining the maximum complexity of neurobehavioral responses and the neural reserve of an organism, are plotted (top) in relation to periods and events occurring during the life span of most mammalian species. Various types of experimental exposure periods are depicted in relation to different points in life when they might be administered to reflect actual types of exposure problems. Toxicity testing points (*), when different types of anatomical and behavioral evaluations are appropriately conducted, are noted in relation to the exposure periods.

Now let us examine how and when toxic agents might exert effects that alter this normal pattern of initial growth, relative stability, and later decline in funcational brain capactiy. Also let us determine appropriate points for testing for such toxic effects. As depicted at the bottom right of Figure 1, during adulthood, mature brain functioning could be affected at various times by exposures of varying durations, ranging from brief acute exposures to somewhat longer subacute or much longer chronic exposures. One must suspect that the geriatric period would be a particulary vulnerable time for the induction of deleterious effects by exposures of any duration, given the likely additive nature of toxic effects with degenerative senescence processes already underway. In regard to testing for toxic effects on behavior, various testing procedures appropriate for adult subjects are available and could be employed at several possible points in relation to different exposure periods, as also indicated in Figure 1. This might first start with establishment of pre-exposure behavioral baselines or, in other cases, such testing might not commence until exposures begin or soon after their termination, provided that appropriate control groups are employed. In addition, if no effects are immediately found upon initial testing at the time of exposure, it should be remembered that certain agents have already been shown to induce progressive damage that would be detectable only by later, delayed testing long after the precipitating exposure had ceased. This suggests that, if initial results are negative, then repeated tests at intervals throughout the rest of the test subjects' lives may be necessary to really be sure that no hidden, long-delayed exposure effects are missed. Again, the geriatric period is likely a highly vulnerable time for such delayed effects to be manifested. Thus, "old age" testing may be especially crucial in seeking out delayed exposure effects, with an ill-defined generalized premature senscence possibly being one syndrome induced by an otherwise apparently harmless agent. It can also be argued that repeated testing at intervals should be conducted even if deficits are found with initial tests, in order to ascertain whether or not the effects observed are permanent or if recovery of function occurs. In sum, the logic just outlined constitutes a general rationale, based on the functional brain capacity concept discussed earlier, which could be applied in planning behavioral toxicology evaluations of adult subjects.

In regard to exposure periods and testing points more specifically of relevance to behavioral teratology studies, such exposure periods are depicted mainly at the bottom left of Figure 1 and include pregestational, prenatal, and neonatal ones, or combinations of all three or even continous whole-life chronic exposures. For exposures occurring before birth, prenatal embryotoxicity-teratology screening at various points during gestation and postnatal developmental testing would be appropriate. The latter would be useful as well for evaluation of effects of exposures started at birth or soon after and should not only include assessment of changes occurring very early neonatally, but also evaluation somewhat later of events associated with the occurrence of puberty. Adult behavioral testing and later geriatric period testing would also be desirable, in order to test for delayed effects appearing during maturity or for the induction of premature senescence by agents passing evaluations carried out earlier in development. Other comments in the preceding paragraph regarding the need for repeated testing at intervals throughout adulthood also apply here. The logic behind the pattern of testing just outlined demands that the same subjects be reevaluated at many points in their lives, thus requiring that longitudinal research designs be employed, as alluded to in Figure 1.

To summarize what is implied by the preceding discussion within the context of the conceptual framework depicted in Figure 1, exposures to toxic agents early in development are seen as possibly exerting three general types of effects on functional brain capacity: (1) slowing or retardation of the normally marked increase in functional capacity early in life, as the brain and the rest of the nervous system undergo maturational development (In essence, this type of effect would constitute a shifting to the right of the initial ascending portion of the curve representing functional capacity in Figure 1); (2) truncation of the total normal growth of functional capacity or lowering of the ultimate maximum level attained at maturity, resulting in decreased complexity of behavior mediated in adulthood or decreased ability to cope under challenging circumstances when one aspect or another of neural functioning is pushed to its limits (Note that this second type of effect need not necessarily be an invariant sequela to the occurrence of the first type of exposure effect, i.e. the induction delayed development); (3) advancement in time or exacerbation of the decline in functional capacity normally seen late in life or the induction of premature senescence, signified by a shift to the left of the last portion of the curve in Figure 1.

(This may result from the acceleration of normal degenerative processes or the addition of still other types of deletarious effects as the residue of early insults.)

The different general categories of evaluations mentioned above, then, are seen as the means by which these three types of effects of early toxic exposures can be effectively demonstrated at appropriate periods following early insults. Prenatal teratology screening and postnatal developmental testing, for example, assess the occurrence of the first type of effect listed. Adult behavioral testing employed later, with the repetition of certain specific tests at intervals, assesses whether the last two types of effects have occurred and helps to evaluate recovery from any deficits found earlier. With the scope of the present problem now better defined and at least some general steps needed to deal with it noted, let us proceed to more specific consideration of different behavioral teratology research strategies that might be applied as toxicity screening approaches.

Clinical Hypothesis Testing Approaches

Some appreciation for the rather large dimensions and compex nature of the task confronting scientists attempting to screen for neurobehavioral toxicity effects can be gained from the discussion of the abstract conceptual framework outlined above. The full enormity of the problem, however, is not brought home completely until one tries to translate the implications of such a theoretical scheme into recommendations for practical action. Questions of importance abound, such as: (1) What species should be used as experimental subjects? (2) What doses and exposure regimens should be utilized for agents to be evaluated? (3) What behaviors or other neural functions should be assessed and in what order? (4) Which specific tests for particular functions should be employed? (5) How does one interpret results from the various tests? (6) What constitutes sufficient evidence of toxicity to warrant the rejection or banning of a particular agent? It is beyond the scope of present purposes to attempt to deal with all of these issues or any signle one in exhaustive detail. Rather, selected aspects of some will be discussed, with the reader being referred to other articles for more detailed information or views on various points.

The issue of what functions should be assessed and in what sequence forms one major focal point for the remaining discussion. In the past, one of the main determinents of which functions were chosen for evaluation was the presentation of clinical or epidemiological evidence that particular functions in humans are affected by a suspected toxin. To illustrate, what will be termed here as a "clinical hypothesis testing" approach is essentially the unstated model that can be discerned as having guided much of the experimental research over the past several years on the neuro-behavioral effects of two heavy metals, mercury (as methylmercury) and lead. Clinical and epidemiological reports of neurological deficits seen in humans and descriptions of symptoms seen in nonlaboratory animals suffering from Minamata disease stimulated experimental screening for particular types of neurobehavioral deficits following methylmercury poisoning, as described by Spyker (*9,13*). Similarly, clinical reports (*14,15*) implicating low level lead exposures as being a possible causitive factor in the etiology of hyperactivity in children have helped to generate numerous animal model evaluations (*16-18*) aimed at assessing that possibility experimentally.

Clinical hypothesis testing approaches of the above type have certainly generated useful information confirming at least some clinically derived suspicions about particular agents and have helped to define threshold doses for induction of toxic effects. Such approaches, however, suffer from the obvious disadvantage of demonstrating deleterious effects after notable damage to human populations has presumably already occurred. In fact, that inadequacy accounts in part for the substantial inpetus behind current efforts to institute new screening approaches designed to detect harmful effects of agents before they are released into the environment. Still, if we use as our guide the wide variety of symptoms reported in humans as being associated with exposure to different toxic agents (*19*), then it very quickly becomes clear that any "pre-release" behavioral screening approach should ideally be very broad or comprehensive, indeed, in regard to the types of functions assessed. This is necessitated by the fact that so many types of functions could be adversely affected by an agent, based on past experience, and yet there is virtually no certain way to predict *a priori* which specific function(s) will be affected by a particular agent. In essence, casting a wide net by means of a comprehensive approach would help to maximize the chances of almost any kind of behavioral deficit being picked up by the screening procedures.

Comprehensive Screening Approaches

Some examples of fairly comprehensive behavioral screening approaches have been outlined in the literature (*5,13*). The former one (*5*), in particular, is spelled out in considerable detail and offers recommendations for following a systematic sequence of discrete steps in evaluating a new agent with unknown, but possibly deleterious neurobehavioral effects. In brief, it proposes initial testing of acute exposure effects on adult subjects, using certain very gross observations (an elementary screen) at first to detect overtly obvious signs of toxicity. After repetition of testing with the "elementary screen" following an interval of time in order to assess delayed effects or recovery from earlier effects, it is recommended that prenatal exposures be carried out and assessment of the offspring by a maturational-development screen be undertaken. Depending upon the outcome of that screen and for other subsequent ones, increasingly more sophisticated behavioral assessments are suggested in order to define even more specific behavioral deficits. After each major evaluation step, decision points are indicated where interpretation of accumulated data leads either to (1) rejection of the agent being tested and cessation of testing or (2) further testing until chronic exposures are finally evaluated, if warranted by anticipated exposure patterns for the agent. A wide variety of behavioral evaluations are suggested as possibly being undertaken as part of the elementary screen, the maturational-develpment screen, or the successively more sensitive later behavioral assessments.

Another comprehensive screening approach, incorporating many features of the sequential analysis described above but designed very specifically for use in behavioral teratology studies, is depicted in Table 1. This complete screening approach assumes prenatal exposure to the agent under evaluation. Also, given that the main advantage of behavioral teratology tests presumbly lies in detecting toxic effects at exposure levels below those producing gross overt toxicity or histologically demonstrable signs of anatomical damage in adult subjects, it is recommended that the present approach be reserved only for use in evaluation of relatively low level exposures to a given agent. That is, only apparently subtoxic exposure levels, as defined first by other screening procedures with exposure of adult subjects, should be employed here, starting with at least the highest apparent subtoxic exposure level. As for evaluations, top priority should initially be assigned to carrying out a so-called "perinatal teratology screen." This screen would involve analyses of embryotoxicity, as well as gross anatomical inspections and/or histological analyses, with special attention accorded the central nervous system, organs of special sense, endocrine organs, and reproductive organs. The term "perinatal" is used here to emphasize that not only should such analyses be carried out on fetuses harvested prior to birth, but also perhaps neonatally during suckling or even a bit later, once proliferation of neural tissue should have been completed. Still later, neuropathology evaluations might also be conducted. Anatomical damage detected by any of the above assessments would argue strongly for rejection of an agent at the offending exposure levels. Lack of effects at this point for given exposures, however, signals the need for further testing.

Further testing would next consist of conducting a postnatal development screen on subjects not sacrificed for teratology screening. The postnatal development screen would evaluate the types of variables listed in Table 1. Evaluations designed to assess the progress of maturation early in development might profitably include not only behavioral tests, but also measures of growth and physical development. This allows for estimates of whether behavioral changes likely indicative of altered neural function occur at exposure levels below those producing general effects on growth or maturation of other organ systems. Thus, ages at which certain physical development landmarks, e.g., incisor eruption, eye opening, descent of testes, and vaginal opening appear, should be recorded. In addition, behavioral assessments should focus early in postnatal development on (1) the maturation of certain reflexes, e.g., righting responses, auditory startle responses, and visual placing response, (2) the shift of immature patterns of locomotion and movement to more adult patterns, and (3) the maturation of other functions, eg. thermoregulatory responses. (More detailed descriptions of possible postnatal development assessments and their interpretation have been given by Rodier (*20*). Stunting of growth, or major delays in physical development or the maturation of particular functions here argue strongly for rejection of an agent, but further testing would still be desirable to ascertain whether or not any recovery of function might occur later in life after various exposures levels.

Table 1. Possible comprehensive behavioral teratology screening approach.[a]

Perinatal Teratology Screen
Central nervous system
Organs of special sense
Endocrine organs
Reproductive system organs
Other organ systems
Postnatal Development Screen
Growth
Physical landmarks
Specific sensory-motor integration reflexes
Fine motor skills
Locomotor patterns
Activity levels
Thermoregulation
Adult Behavioral Testing
Preliminary Screen
Body weight changes
Objective signs
Reflex changes
Elicited responses
Gross motor changes
Body temperature
Intermediate testing
Consummatory responses
Activity patterns
Motor performance
Sensory perception
Sex/maternal behavior
Emotionality/aggression
Advanled testing
Classical conditioning
Operant conditioning
(Appetitive/aversive)
Memory processes
Sensory capabilities
Fine motor skills

[a] Assuming a prenatal exposure period, evaluations would proceed in the following sequence: (1) perinatal teratology screening; (2) postnatal development screening; (3) adult behavior testing, proceeding from the preliminary screen through intermediate testing to advanced testing, if necessary.

Conversely, failure to find effects here or detection of apparently minor developmental delays does not necessarily establish the safety of an agent. Rather, further testing would be needed later in adulthood or in old age to determine possible delayed effects.

Adult behavioral testing might then proceed in a sequence consisting of increasingly more sophisticated and difficult assessment procedures. The first, and grossest level of assessment, would consist of continuous monitoring for overt signs of toxicity. Elementary or preliminary screens often already used by pharmaceutical houses, would be employed for this purpose. Observations usually requiring little more than standardized ratings and minimum equipment are made on the types of variables listed for this first screening level. Objective signs include general physical appearance, e.g., scruffiness of coat, abnormal postures, excessive salivation or diarrhea, ptosis of the eyelid, and so on. Elicited responses include reactions to handling, reactions to nose or tail pinch, etc. Reflex changes included alterations in righting responses, corneal or visual placing responses, and auditory startle, among others. Observations of gross motor changes include abnormal circling, obstinate progression, hyper-or hypoactivity during brief observation, and lack of grasping reflexes. Presumably, toxic effects so grossly manifested as to be detected by these procedures would be considered to be severe enough to justify cessation of testing and probable rejection of the agent tested. Failure to observe reliable effects here, however, by no means comes close to establishing the nontoxicity of the agent at exposure levels studied; rather, still more sophisticated levels of behavioral testing would be recommended for use.

The next or "intermediate" level of adult behavioral testing would involve attempts at quantifying changes in spontaneous behavioral responses, e.g., monitoring locomotor activity, food and water consumption, and also relatively gross measures of motor performance or sensory perception. The third or "advanced" level of assessment, however, involves analyses of behavior in much more controlled circumstances and would include tasks such as operant conditioning paradigms aimed at assessing an agent's impact on learning and memory functions and/or to detect even very small shifts in sensory capabilities or fine motor skills. Weiss (5) lists specific types of tests that might be used at this level of analysis. Some functions, e.g., sleep, that require electrophysiological assessments have not been included here, but their evaluation would also obviously be desirable. Given that delayed effects of insults early in development may not be manifested until late in adult life, one or more components of the present adult behavior testing procedure may need to be repeated, especially as subjects begin to enter old age, in order to detect induction of premature senscence.

Comprehensive testing approaches of the kind outlined above would allow for a broad and detailed description of myriad neurobehavioral effects induced by a given agent at various points in life. They would, however, be very costly and time-consuming to conduct, with final results not available possibly for several years, depending on the animal species used and whether testing

during the geriatric life period became necessary in the face of lack of effects seen with early screens. In fact, if a longitudinal approach of the above type were to be applied in its entirity as a routine "pre-release" screening program, then the choice of test subjects would perforce be realistically limited to species, such as mice or rats, with life spans of less than 3-4 years. Even with such test subjects, however, the likelihood exists that is could take at up to 5 years to complete a thorough, comprehensive evaluation of the neurobehavioral effects of a given agent, especially if detailed data were sought regarding the types of subtle behavioral deficits most likely to occur after exposures to otherwise apparently subtoxic low levels of the agent. Detection of subtle behavioral changes often require use of the most sophisticated advanced behavioral testing techniques available and even then they might not be detectable until well into old age. Thus, the cost in elapsed time, not to mention the commitment of laboratory space, trained personnel, and expensive equipment, can be expected to be quite considerable if an all-out effort is undertaken to identify the lowest exposures at which an agent exerts even very minute neurobehavioral effects.

A legitimate question arises, in view of the above factors, as to whether the gain expected really justifies the immense costs associated with carrying out comprehensive screening approaches of the kind under discussion. In part, this may depend upon the anticipated benefits to be derived from use of the agent evaluated, which is a factor helping to determine just how far one might choose to proceed through a sequence of increasingly more difficult analyses of neurobehavioral effects (see 5 for more information dealing with this point). Inronically, even if the entire testing sequence is completed, one cannot be completely certain that no important effect has been missed, as suggested elsewhere (*6,12*). Nor is it always easy to extrapolate with confidence from animal findings as to what their significance might be in regard to human neurobehavioral functions or the exposure levels needed to induce a given defict in humans (*12,20*).

The above points could be construed as arguing for the complete abandonment of any attempt at large-scale neurobehavioral toxicity screening, given the resources demanded, the possibility of still missing important deficits, and difficulties at times in interpreting results and their significance for predicting human exposure effects. Quite to the contrary, despite these difficulties, comprehensive screening programs of the type outlined above likely do presently represent the single best appraoch available to maximize the probability that subtle neurobehavioral toxicity effects will be detected and defined with some precision; still, it would obviously be desirable if alternative screening heuristics could be developed that would provide savings in time and resources needed and yet not badly sacrifice the likelihood of detecting significant neurobehavioral deficits. Some possibilities for moving in this direction are briefly outlined below.

Alternative Screening Heuristics

Many possibilities exist for the development of effective screening approaches as alternatives to comprehensive, broad spectrum screening programs. Most, however, require that we be willing to accept substantially less detailed information than we might ideally like to have on the neurobehavioral effects of a given agent before recommending its acceptance or rejection for release into the environment.

One alternative approach is to try to narrow down judiciously the range of functions assayed for a particular agent, i.e., to set priorities for particular types of neurobehavioral tests to be carried out. The priorities set may be agent-specific, in order to maximize the chance that a given agent's most likely neurobehavioral effects will be picked up. For example, if an agent is chemically closely similar to a class of other toxic substances known to cause deficits in certain neurobehavioral functions, but not others, then screening efforts for the agent under study might be best concentrated on those functions affected by the analogous substances. Another basis on which priorities for conduction of particular types of screening tests might be set would not be agent-specific; rather, it would consist of relegating to lowest priorities assessments of particular functions for which the available testing method ologies are not well established or the results of which are difficult to interpret or extrapolate to humans. Conversely, highest priorities could be assigned to functions for which the evaluation methodology is well worked out and produces results that have generally been found to be easily extrapolated to humans. Contrast, for example, the current state of affairs in regard to the assaying of aggressive behaviors, described recently (*21*) as being fraught with difficulties in interpretation of results and in extrapolation of the likely meaning of results in so far as they might apply to humans, with the much

better established and accepted methodology for assaying sensory functions, which also yield results often fairly directly extrapolatable to humans (*22,23*). In other words, perhaps it would be best to set priorities so as to emphasize screening efforts that are capable at the time of yielding solid, interpretable data, which would well define particular neurobehavioral deficits if they were present. On the other hand, if we are willing to accept less well-defined or seemingly innocuous changes as indicators of likely altered functional brain capacity and, therefore, potential toxicity, then several other screening heuristics may well prove to be useful screening short-cuts.

The key features of three such alterative approaches now under investigation as to their potential usefulness for facilitating screening of neurobehavioral deficits after environmental insults early in development are: (1) assessment of developmental delays as predictors of subsequent adult neurobehavioral deficits; (2) behavioral assessments by use of apical test paradigms that simultaneously monitor multiple functions; (3) employment of provocative drug tests as probes for altered functional brain capacity. Each shows promise of possibly shortening considerably the time and effort needed to demonstrate effects which likely constitute danger signals indicating that an agent has or will later exert a significant damaging effect on neurobehavioral functioning. In regard to the first alternative, substantial time could be saved in future behavioral teratology screening efforts, if it were possible to show convincingly that early delays in physical or behavioral development, no matter how small, transient, or seemingly insignificant in and of themselves, nevertheless are highly predictive of more serious later adult neurobehavioral deficits to come. If further research can establish this as a general rule, then finding a developmental delay relatively early in a behavioral teratology testing sequence may be sufficient to warrant cessation of further testing and immediate rejection of any agent having little or no potential use value. Temporary withholding of other agents having greater potential benefits might also be warranted, pending more thorough analyses of any neurobehavioral deficits that appear after early delays in development.

The logic behind use of apical test paradigms, was spelled out in part by Butcher (*6*). Briefly, the main idea is to concentrate on developing behavioral test paradigms that tap or assay multiple neurobehavioral functions such that a single test situation might be used in lieu of several other types of evaluations aimed at assessing single functions in detail. Successful performance in many complex operant conditioning paradigms, for example, typically depends on multiple neurobehavioral processes being intact. Thus, deficits in performance in such situations may be due to alterations in sensory or motor functions, motivation, or learning and memory processes. To be certain which of these processes has been affected can require extensive additional testing to pin down the exact source of the performance deficit. However, if we are willing simply to accept any alteration in performance in such apical test situations as being indicative of altered functional brain capacity after environmental insult without going on to elucidate the exact nature of the deficit, then this may form a basis for more rapid screening of agents.

The last alternative, the use of provcative drug tests as probes for altered functional brain capacity, is based on an extensive psychopharmacology literature demonstrating that various types of damage to the CNS result in increased or decreased sensitivity to various drugs. Testing for paradoxical responses to amphetamine after low level lead exposures (*17,18*) represents one example of employment of this type of screening heuristic. Such provocative drug probes could be used at various points after environmental insults early in development, as schematically illustrated in Figure 1. More research is needed to establish whether altered response to drugs, especially early in postnatal development, are reliable predictors of other more serious prenatal insult induced neurobehavioral deficits seen later in life. If so, then such provocative drug tests may represent yet another way of substantially reducing the time and effort needed in order to demonstrate sufficient evidence of danger of neurobehavioral toxicity to reject an agent early in a screening sequence.

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